

REMARKS / ARGUMENTS

It is noted that in view of Applicant's amendments and remarks filed on May 19, 2005, all of the previous objections and rejections have been withdrawn. The following new rejections are based on a new primary reference, namely the van Reis reference that Applicant had disclosed in the Information Disclosure Statement, combined with previously cited references. Claims 1-16 are pending and at issue.

Claim Rejections – 35 USC § 103(a)

Claims 1-16 stand rejected as allegedly being obvious over the combination of van Reis (*Biotechnology and Bioengineering*, Vol. 38, p. 413-422, 1991) and U.S. Patent No. 5,780,601 granted to Green et al. ("the Green '601 patent").

Claims 1-16 are directed to separate processes for extracting native or recombinantly-expressed, gram-negative inner (claims 1-3 and 6) and outer (claims 4, 5 and 7-16) membrane proteins from bacteria or bacterial host cells by differential detergent tangential flow diafiltration. The extraction process for outer membrane proteins is exemplified with the recombinant forms of the P4 and P6 proteins of *Haemophilus influenzae*, as expressed in an *E. coli* host cell. Specifically, the process for the recombinant P4 protein includes the steps of:

- (a) lysing bacteria or bacterial host cells containing a recombinant vector in a fermentation broth;
- (b) diafiltering the lysed fermentation broth from (a) with a buffer which is not retained by the diafiltration membrane, wherein said buffer removes intracellular and extracellular contaminants through the permeate, and using a chelating agent to prevent proteolysis;
- (c) diafiltering the lysate from (b) with a detergent and a buffer which is not retained by the diafiltration membrane, wherein said detergent solubilizes and removes inner membrane proteins, and using a divalent cation to stabilize the outer membrane proteins, thereby preventing their solubilization;

- (d) diafiltering the lysate from (c) with the buffer from (c), and using a divalent cation from (c) in the absence of detergent, in order to reduce the concentration of the detergent from (c);
- (e) diafiltering the lysate from (d) with a buffer which is not retained by the diafiltration membrane, a chelating agent and a detergent to solubilize and remove the outer membrane proteins; and
- (f) collecting the outer membrane proteins removed in (e).

The claimed invention is thus directed to a process for the purification of bacterial membrane proteins that combines chemical extraction and the use of tangential flow filtration methods. This novel combination allows for the predictable and economical scale-up of these methods to a commercial manufacturing scale.

The claimed process is an improvement over alternate processes, such as centrifugation, in that it provides added selectivity to the process, i.e., not only must the proteins be soluble, they must also have the ability to pass through the diafiltration membrane, which has a defined size cut-off or opening. The sequence of buffer solutions is chosen to solubilize inner membrane proteins first and then to solubilize the outer membrane proteins. During diafiltration, the solubilized proteins of approximate size less than the molecular weight cut-off of the membrane pass through with the permeate, while larger molecules and unsolubilized proteins are retained.

The van Reis article is a simplistic restatement of the fact that tangential flow filtration (TFF) systems are used to separate materials, protein or otherwise, according to molecular weight. This type of information is available from any TFF membrane manufacturer. Even the International Search Report categorizes the van Reis article as "A" – a document defining the general state of the art that is not considered to be of particular relevance. The van Reis article does not disclose or suggest the combination of tangential flow filtration methods and the use of detergent extraction for the purification of membrane proteins. Thus, the Examiner turns to the Green '601 patent to fill the gap.

The Green '601 patent discloses a method of purifying P4 by differential detergent extraction utilizing physical processing methods such as differential sedimentation (col. 4), gradient sedimentation (col. 4), and centrifugation (col. 14), none of which rely on molecular size differences. The only requirement of these processes is

that the membrane proteins be either soluble or insoluble; these proteins are not required to pass through a diafiltration membrane. Nonetheless, the Examiner asserts that it would have been *prima facie* obvious at the time the invention was made to use the tangential flow filtration taught by van Reis et al. to extract bacterial inner and outer membrane proteins because the benefits of TFF include, among other things, the potential for linear scale-up and the ability to be used for a large number of products without additional development work.

Applicant traverses because the Examiner's rejection lacks the necessary substantial evidence to support a rejection of Applicant's claims.

Most if not all inventions arise from a combination of old elements. *See In re Rouffet*, 149 F.3d 1350, 1357, 47 USPQ2d 1453,1457 (Fed. Cir. 1998). Thus, every element of a claimed invention may often be found in the prior art. *See id.* However, identification in the prior art of each individual part claimed is insufficient to defeat patentability of the whole claimed invention. *See id.* Rather, to establish obviousness based on a combination of elements disclosed in the prior art, there must be some motivation, suggestion or teaching of the desirability of making the specific combination that was made by the applicant. *See In re Dance*, 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998); *In re Gordon*, 733 F.2d 900, 902, 221 USPQ 1125, 1127 (Fed. Cir. 1984). It is impermissible to use the claims as a framework from which to pick and choose among individual references to recreate the claimed invention. *See In re Fine*, 5 USPQ2d 1586, 1600 (Fed. Cir. 1988).

The motivation, suggestion or teaching may be found in explicit or implicit teachings within the references themselves, from the ordinary knowledge of those skilled in the art, or from the nature of the problem to be solved. *See WMS Gaming, Inc. v. International Game Tech.*, 51 USPQ2d 1385, 1397 (Fed. Cir. 1999). However, there still must be **evidence** that "a skilled artisan, confronted with the same problems as the inventor and with no knowledge of the claimed invention, would select the elements from the cited prior art references for combination in the manner claimed." *In re Rouffet*, 47 USPQ2d at 1456; *see also In re Kotzab*, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000) ("[a] rejection cannot be predicated on the mere identification . . . of individual components of claimed limitations. Rather, particular findings must be made as to the reason the skilled artisan, *with no knowledge of the claimed invention*, would have selected these components for combination in the manner claimed.") (Emphasis added.)

Here, no such evidence was presented. Rather than pointing to specific information in the Green '601 patent that suggests its combination with van Reis et al. to yield the claimed invention, the Examiner merely discusses how the cited references can be combined to read on the claimed invention. This reference-by-reference, limitation-by-limitation analysis wholly fails to demonstrate how the cited references teach or suggest the combination claimed in the present invention. *In re Dembiczak*, 50 USPQ2d 1614, 1618 (Fed. Cir. 1999).

Essentially, the Green '601 process consists of mostly batch-wise centrifugation steps – which the present invention avoids – that are not economically feasible for manufacturing scale processes. Consequently, combining the tangential flow filtration of van Reis with the physical processing methods of the Green '601 patent does not yield the invention as claimed, i.e., a process that combines chemical extraction and tangential flow filtration. Thus, the rejection is improper and should be withdrawn.

Claims 1-16 also stand rejected as allegedly being obvious over the van Reis article in view of Anilionis et al. (*U.S. Patent No. 5,098,997*) and further in view of Kolbe (*U.S. Patent No. 5,276,141*).

The presently claimed invention and van Reis et al. were described above.

Anilionis et al. disclose a method of isolating and purifying outer membrane proteins P4 and P6 by differential detergent extraction utilizing sonication and centrifugation (col. 26-27), neither of which relies on molecular size differences. As noted above, the only requirement of these processes is that the membrane proteins be either soluble or insoluble; these proteins are not required to pass through a diafiltration membrane. And adding Kolbe to the mix does not erase this flaw.

Kolbe proposes a laboratory scale method of purifying glycosylated proteins by precipitating proteins with divalent metal ions and then centrifuging out the precipitate. Kolbe is not even remotely related to the claimed process other than the fact the proteins are being purified.

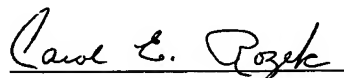
The Examiner asserts that it would have been obvious at the time the invention was made to add the divalent metal ions as taught by Kolbe to the process of extracting proteins by tangential flow filtration as taught by van Reis and Anilionis et al. combined to obtain the claimed invention.

Applicant respectfully disagrees. The claimed invention is a process for the purification of bacterial membrane proteins that combines chemical extraction and the use of tangential flow filtration methods. Focusing on the buffers, detergents, and divalent metal ions common to both the claimed process and those cited in the references is not looking at the invention as a whole, which is a large scale extraction process that avoids the centrifugation of Anilionis et al. and obtains quantities of protein sufficient for economical manufacturing. Even if there were motivation to combine elements of the cited references as the Examiner did, combining the tangential flow process of van Reis with the centrifugation process of Anilionis et al. and the divalent metal ions of Kolbe cannot possibly result in the differential detergent tangential flow diafiltration process as claimed. Thus, Applicant's invention cannot be *prima facie* obvious.

Based on the foregoing, Applicants submit that this rejection based on the combination of van Reis, Anilionis et al. and Kolbe is improper and should be withdrawn.

In view of the above remarks, Applicants submit that the present application is in condition for allowance, and a Notice to that effect is requested.

Respectfully submitted,



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